Cyclo(RGDyK)-Cy5.5
RGD-Cy5.5

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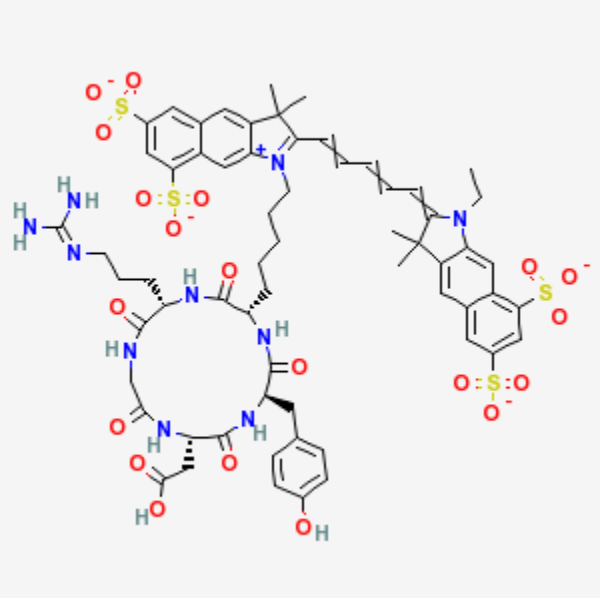
Chemical name: Cyclo(RGDyK)-Cy5.5
Abbreviated name: RGD-Cy5.5
Synonym:
Backbone: Peptide
Target: Integrin $\alpha_v\beta_3$
Mechanism: Receptor binding
Method of detection: Optical, near-infrared
Source of signal: Cy5.5
Activation: No
***In vitro* studies:** Yes
Rodent studies: Yes
Other non-primate mammal studies: No
Non-human primate studies: No
Human studies: No



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Background

[PubMed]

Integrins are a family of cell surface heterodimeric glycoproteins that mediate diverse biological events involving cell-cell and cell-matrix interactions (1). They consist of an α and a β subunit. They are important for cell adhesion and signal transduction. The $\alpha_v\beta_3$ integrin is the most prominent receptor class affecting tumor growth, tumor invasiveness, metastasis, tumor-induced angiogenesis, inflammation, osteoporosis, and rheumatoid arthritis (2-7). The $\alpha_v\beta_3$ integrin is strongly expressed on tumor cells and activated endothelial cells. In contrast, expression of $\alpha_v\beta_3$ integrin is weak on resting endothelial cells and most normal tissues. The $\alpha_v\beta_3$ antagonists are being studied as anti-tumor and anti-angiogenic agents (4, 8, 9) and the agonists as angiogenic agents for coronary angiogenesis (10, 11). A tripeptide sequence consisting of Arg-Gly-Asp (RGD) is identified as

a recognition motif used by extracellular matrix proteins (vitronectin, fibrinogen, laminin, and collagen) to bind to a variety of integrins including $\alpha_v\beta_3$. Various radiolabeled antagonists were introduced for imaging of tumors and tumor angiogenesis (12).

Optical fluorescence imaging is increasingly used to obtain biological functions of specific targets (13, 14). However, the intrinsic fluorescence of biomolecules poses a problem when visible light (350-700 nm) absorbing fluorophores are used. Near-infrared (NIR) fluorescence (700-1000 nm) detection avoids the background fluorescence interference of natural biomolecules, providing a high contrast between target and background tissues. NIR fluorophores have wider dynamic range and minimal background as a result of reduced scattering compared with visible fluorescence detection. They also have high sensitivity, resulting from low infrared background, and high extinction coefficients, which provide high quantum yields. The NIR region is also compatible with solid-state optical components, such as diode lasers and silicon detectors. NIR fluorescence imaging is becoming a non-invasive alternative to radionuclide imaging.

Cyclo(RGDyK) was conjugated with Cy5.5 to study *in vivo* biodistribution of the tracer in tumor-bearing mice. Cy5.5 is a NIR fluorescent dye with absorbance maximum at 675 nm and emission maximum at 694 nm with a high extinction coefficient of 250,000 $(mol/L)^{-1}cm^{-1}$. RGD-Cy5.5 or c(RGDyK)-Cy5.5 was found to have a high and long-lasting accumulation in $\alpha_v\beta_3$-positve U87MG human glioblastoma tumor cells in nude mice (15). The binding of RGD-Cy5.5 to the integrin receptor was found to be specific both *in vitro* and *in vivo*.

Synthesis

[PubMed]

Cy5.5 monofunctional *N*-hydroxysuccinimide (NHS) ester was used to conjugate c(RGDyK) to form c(RGDyK)-Cy5.5, which was purified by high-performance liquid chromatography (15). A chemical yield of 70-75% was obtained. The NHS ester of Cy5.5 is reacted with the ε-amino group of the lysine in c(RGDyK). The peak containing the RGD-Cy5.5 conjugate was analyzed by matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS). The measured mass (*m*/*z*, 1518.4) was the same as the expected mass (*m*/*z*, 1518.7).

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

A receptor-binding assay with ^{125}I-labeled c(RGDyK) (0.06 nM) was established using immobilized $\alpha_v\beta_3$ receptors on plastic microtiter plate wells. RGD-Cy5.5 and c(RGDyK) had an IC_{50} of 58.1 and 37.5 nM, respectively (15). Receptor-mediated endocytosis of RGD-Cy5.5 in U87MG tumor cells and HBCEC human brain endothelial cells was observed by confocal laser-scanning microscopy. Binding of 100 nM RGD-Cy5.5 to both cell types was completely blocked by 10 μM c(RGDyK).

Animal Studies

Rodents

[PubMed]

Biodistribution studies of RGD-Cy5.5 were evaluated in nude mice using a U87MG glioblastoma subcutaneous xenograft model (15). U87MG tumor cells were injected subcutaneously into the right foreleg. Images were obtained at 10 min, 30 min, 1 h, 2 h, 4 h, and 24 h after injection of 0.5 nmol of RGD-Cy5.5. The tumor uptake of RGD-Cy5.5 could be clearly seen from 30 min to 24 h. A maximal uptake in the tumor was reached at 2 h and slowly washed out over time. The tracer uptake could be blocked by co-injection of 100 nmol c(RGDyK). This proves to be a non-invasive imaging of tumor cells in mice by using optical technique.

Other Non-Primate Mammals

[PubMed]

No publication is currently available.

Non-Human Primates

[PubMed]

No publication is currently available.

Human Studies

[PubMed]

No publication is currently available.

References

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